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L8 ANSWER 1 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2003-278642 [27] WPIDS
DOC. NO. CPI: C2003-072953
TITLE: New purified peptide comprising a variant proaerolysin amino acid sequence with a prostate-specific **protease** cleavage site and a functionally deleted furin cleavage site, useful for treating prostate cancer.
DERWENT CLASS: B04 D16
INVENTOR(S): BUCKLEY, J T; DENMEADE, S R; ISAACS, J T
PATENT ASSIGNEE(S): (UYJO) UNIV JOHNS HOPKINS; (UYVI-N) UNIV VICTORIA INNOVATION & DEV CORP
COUNTRY COUNT: 101
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2003018611	A2	20030306	(200327)*	EN	83

RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003018611	A2	WO 2002-US27061	20020823

PRIORITY APPLN. INFO: US 2001-314613P 20010824
AN 2003-278642 [27] WPIDS
AB WO2003018611 A UPAB: 20030429
NOVELTY - A purified peptide comprising a variant proaerolysin amino acid sequence having a prostate-specific **protease** cleavage site and a functionally deleted furin cleavage site, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) treating prostate cancer by administering the peptide or the nucleic acid encoding the peptide to the subject, or contacting prostate cancer cells of the subject with the peptide;

(2) systematically treating prostate cancer in a subject by removing prostate cancer cells from the subject, contacting the cells with the peptide to generate a cell lysate, and administering the cell lysate to the subject; a purified peptide comprising a variant proaerolysin amino acid sequence comprising a furin cleavage site, a functionally deleted proaerolysin binding domain, and a prostate-tissue specific binding domain;

(3) a purified peptide comprising a variant **Clostridium** septicum alpha **toxin** amino acid sequence comprising a prostate-specific **protease** cleavage site and a functionally deleted furin cleavage site;

(4) a purified peptide comprising a variant Bacillus thuringiensis delta-**toxin** amino acid sequence comprising a prostate-specific **protease** cleavage site and a functionally deleted wild type activation sequence;

(5) a purified peptide comprising a variant human perforin amino acid sequence comprising prostate-specific **protease** cleavage site and a functionally deleted wild type perforin activation site; a purified peptide comprising a variant **Clostridium** septicum alpha **toxin** amino acid sequence comprising a furin cleavage site, a functionally deleted **Clostridium** septicum alpha **toxin** binding domain, and a prostate-tissue specific binding domain;

(6) a purified peptide comprising a variant Bacillus thuringiensis delta-**toxin** amino acid comprising wild type activation site, a functionally deleted Bacillus thuringiensis delta-**toxin** binding domain, and a prostate-tissue specific binding domain; a purified peptide comprising a variant human perforin amino acid sequence comprising a wild type activation site, a functionally deleted perforin binding domain, and a prostate-tissue specific binding domain; and

(7) a nucleic acid sequence encoding the peptide.

ACTIVITY - Cytostatic.

No biological data given.

MECHANISM OF ACTION - Peptide therapy.

USE - The peptides and **methods** are useful for treating prostate cancer (claimed).

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L8 ANSWER 1 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN

ACCESSION NUMBER: 2003-278642 [27] WPIDS

DOC. NO. CPI: C2003-072953

TITLE: New purified peptide comprising a variant proaerolysin amino acid sequence with a prostate-specific **protease** cleavage site and a functionally deleted furin cleavage site, useful for treating prostate cancer.

DERWENT CLASS: B04 D16

INVENTOR(S): BUCKLEY, J T; DENMEADE, S R; ISAACS, J T

PATENT ASSIGNEE(S): (UYJO) UNIV JOHNS HOPKINS; (UYVI-N) UNIV VICTORIA INNOVATION & DEV CORP

COUNTRY COUNT: 101

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2003018611	A2	20030306	(200327)*	EN	83

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W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003018611	A2	WO 2002-US27061	20020823

PRIORITY APPLN. INFO: US 2001-314613P 20010824

AN 2003-278642 [27] WPIDS

AB WO2003018611 A UPAB: 20030429

NOVELTY - A purified peptide comprising a variant proaerolysin amino acid sequence having a prostate-specific **protease** cleavage site and a

functionally deleted furin cleavage site, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) treating prostate cancer by administering the peptide or the nucleic acid encoding the peptide to the subject, or contacting prostate cancer cells of the subject with the peptide;

(2) systematically treating prostate cancer in a subject by removing prostate cancer cells from the subject, contacting the cells with the peptide to generate a cell lysate, and administering the cell lysate to the subject; a purified peptide comprising a variant proaerolysin amino acid sequence comprising a furin cleavage site, a functionally deleted proaerolysin binding domain, and a prostate-tissue specific binding domain;

(3) a purified peptide comprising a variant **Clostridium** septicum alpha **toxin** amino acid sequence comprising a prostate-specific **protease** cleavage site and a functionally deleted furin cleavage site;

(4) a purified peptide comprising a variant Bacillus thuringiensis delta-**toxin** amino acid sequence comprising a prostate-specific **protease** cleavage site and a functionally deleted wild type activation sequence;

(5) a purified peptide comprising a variant human perforin amino acid sequence comprising prostate-specific **protease** cleavage site and a functionally deleted wild type perforin activation site; a purified peptide comprising a variant **Clostridium** septicum alpha **toxin** amino acid sequence comprising a furin cleavage site, a functionally deleted **Clostridium** septicum alpha **toxin** binding domain, and a prostate-tissue specific binding domain;

(6) a purified peptide comprising a variant Bacillus thuringiensis delta-**toxin** amino acid comprising wild type activation site, a functionally deleted Bacillus thuringiensis delta-**toxin** binding domain, and a prostate-tissue specific binding domain; a purified peptide comprising a variant human perforin amino acid sequence comprising a wild type activation site, a functionally deleted perforin binding domain, and a prostate-tissue specific binding domain; and

(7) a nucleic acid sequence encoding the peptide.

ACTIVITY - Cytostatic.

No biological data given.

MECHANISM OF ACTION - Peptide therapy.

USE - The peptides and **methods** are useful for treating prostate cancer (claimed).

Dwg.0/5

L8 ANSWER 2 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2003:113075 BIOSIS

DOCUMENT NUMBER: PREV200300113075

TITLE: Fluorigenic substrates for the **protease activities** of botulinum neurotoxins, serotypes A, B, and F.

AUTHOR(S): Schmidt, James J. (1); Stafford, Robert G.

CORPORATE SOURCE: (1) Toxinology and Aerobiology Division, United States Army Medical Research Institute of Infectious Diseases, 1425 Porter St., Fort Detrick, MD, 21702-5011, USA: james.schmidt@det.amedd.army.mil USA

SOURCE: Applied and Environmental Microbiology, (January 2003, 2003) Vol. 69, No. 1, pp. 297-303. print. ISSN: 0099-2240.

DOCUMENT TYPE: Article

LANGUAGE: English

AB The seven botulinum neurotoxins (BoNTs) are zinc metalloproteases that cleave neuronal proteins involved in neurotransmitter release and are among the most toxic natural products known. High-throughput BoNT assays are needed for use in antibotulinum drug discovery and to characterize

BoNT **protease activities**. Compared to other **proteases**, BoNTs exhibit unusually stringent substrate requirements with respect to amino acid sequences and polypeptide lengths. Nonetheless, we have devised a strategy for development of fluorigenic BoNT **protease** assays, based on earlier structure-function studies, that has proven successful for three of the seven serotypes: A, B, and F. In synthetic peptide substrates, the P1 and P3' residues were substituted with 2,4-dinitrophenyl-lysine and S-(N-(4-methyl-7-dimethylamino-coumarin-3-yl)-carboxamidomethyl)-cysteine, respectively. By monitoring the BoNT-catalyzed increase in fluorescence over time, initial hydrolysis rates could be obtained in 1 to 2 min when BoNT concentrations were 60 ng/ml (about 1 nM) or higher. Each BoNT cleaved its fluorigenic substrate at the same location as in the neuronal target protein, and kinetic constants indicated that the substrates were selective and efficient. The fluorigenic assay for BoNT B was used to characterize a new competitive inhibitor of BoNT B **protease activity** with a Ki value of 4 muM. In addition to real-time **activity** measurements, **toxin** concentration determinations, and kinetic studies, the BoNT substrates described herein may be directly incorporated into automated high-throughput assay systems to screen large numbers of compounds for potential antibotulinum drugs.

L8 ANSWER 3 OF 27 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 1

ACCESSION NUMBER: 2002:353597 CAPLUS
DOCUMENT NUMBER: 136:365216
TITLE: Recombinant light chains of botulinum neurotoxins and light chain fusion proteins for use in research and clinical therapy
INVENTOR(S): Smith, Leonard A.; Jensen, Melody
PATENT ASSIGNEE(S): United States Army Medical Research and Material Command, USA
SOURCE: PCT Int. Appl., 166 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002036758	A2	20020510	WO 2001-US47230	20011106
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
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US 2003009025	A1	20030109	US 2001-910186	20010720
AU 2002028887	A5	20020515	AU 2002-28887	20011106

PRIORITY APPLN. INFO.:

US 2000-246774P	P	20001106
US 2001-910186	A	20010720
US 2001-311966P	P	20010809
US 1999-133865P	P	19990512
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US 1999-133873P	P	19990512
US 1999-146192P	P	19990729

WO 2000-US12890 W 20000512
US 2000-611419 A1 20000706
WO 2001-US47230 W 20011106

AB Botulinum neurotoxins, the most potent of all **toxins**, induce lethal neuromuscular paralysis by inhibiting exocytosis at the neuromuscular junction. The light chains (LC) of these dichain neurotoxins are a new class of zinc-endopeptidases that specifically cleave the synaptosomal proteins, SNAP-25, VAMP, or syntaxin at discrete sites. The present invention relates to the construction, expression, purifn., and use of synthetic or recombinant botulinum neurotoxin genes. For example, a synthetic gene for the LC of the botulinum neurotoxin serotype A (BoNT/A) was constructed and overexpressed in Escherichia coli. The gene product was purified from inclusion bodies. The **methods** of the invention can provide 1.1 g of the LC per L of culture. The LC product was stable in soln. at 4.degree. for at least 6 mo. This rBoNT/A LC was proteolytically active, specifically cleaving the Glu-Arg bond in a 17-residue synthetic peptide of SNAP-25, the reported cleavage site of BoNT/A. Its calcd. catalytic efficiency kcat/Km was higher than that reported for the native BoNT/A dichain. Treating the rBoNT/A LC with mercuric compds. completely abolished its **activity**, most probably by modifying the cysteine-164 residue located in the vicinity of the active site. About 70% **activity** of the LC was restored by adding Zn2+-free, apo-LC prepn. The LC was nontoxic to mice and failed to elicit neutralizing epitope(s) when the animals were vaccinated with this protein. In addn., injecting rBoNT/A LC into sea urchin eggs inhibited exocytosis-dependent plasma membrane resealing.

L8 ANSWER 4 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2002-590661 [63] WPIDS
DOC. NO. CPI: C2002-167115
TITLE: **Methods** for surface display of proteins, e.g. displaying a protein on the surface of spores, improving a protein or isolating a substance, by transforming a host cell harboring a genetic carrier with spore or virus with the vector library.
DERWENT CLASS: B04 C06 D16
INVENTOR(S): BAN, J G; CHOI, S G; JUNG, H C; CHOI, S K; PAN, J G
PATENT ASSIGNEE(S): (GENO-N) GENOFOCUS CO LTD
COUNTRY COUNT: 96
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002055561	A1	20020718	(200263)*	EN	118

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

KR 2002061218 A 20020724 (200305)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002055561	A1	WO 2002-KR59	20020115
KR 2002061218	A	KR 2001-2156	20010115

PRIORITY APPLN. INFO: KR 2001-2156 20010115
AN 2002-590661 [63] WPIDS

AB WO 200255561 A UPAB: 20021001
NOVELTY - **Methods** for preparing a protein surface-displayed on a genetic carrier, improving a protein or isolating a substance in a mixture comprising transforming a host cell harboring a genetic carrier consisting of a spore or virus with the vector library, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for:

(1) preparing a protein surface displayed on a genetic carrier comprising:

(a) transforming a host cell harboring the genetic carrier consisting of spore or virus with a vector containing a gene encoding the protein;

(b) culturing the transformed host cell and expressing the protein in the host cell; and

(c) allowing to form non-covalent bonds between the expressed protein and a surface of the genetic carrier so that the protein is displayed on the surface of the genetic carrier;

(2) improving a protein comprising:

(a) constructing a gene library of the protein by means of mutating the gene encoding the protein;

(b) preparing a vector library containing the constructed gene library;

(c) employing step (1-a) and (1-b) above;

(d) obtaining a genetic carrier library by means of allowing to form noncovalent bonds between the expressed protein variant and a surface of the genetic carrier so that the variant is displayed on the surface of the genetic carrier; and

(e) screening the genetic carrier displaying on its surface the variant of the protein having a desired property;

(3) isolating a substance in mixture comprising:

(a) constructing a gene library encoding a variant of binding protein or its binding domain by means of mutating the gene encoding the binding protein or binding domain as protein of interest;

(b) employing (2-b) or (1-a);

(c) obtaining a genetic carrier library by means of allowing to form noncovalent bonds between the expressed binding protein variant or binding domain and a surface of the genetic carrier so that the variant is displayed on the surface of the genetic carrier;

(d) contacting the genetic carrier library with a predetermined substance and screening an improved binding protein or its binding domain by means of selecting the genetic carrier displaying on its surface the variant binding the predetermined substance; and

(e) contacting the genetic carrier displaying on its surface the improved binding protein or its binding domain with the mixture to isolate the substance in mixture;

(4) a vector for displaying on the surface of a genetic carrier a protein, which comprises a replication origin, an antibiotic-resistance gene, a restriction site, or a gene encoding the protein, where the protein, when expressed in a host cell, is capable of forming noncovalent bond to the surface of genetic carrier;

(5) a microbial transformant, which is produced by transforming a host cell harboring spores or viruses with the vector;

(6) a complex between genetic carrier and protein, characterized in that the complex is prepared by displaying on the surface of the genetic carrier, hormone, hormone analogue, enzyme, enzyme inhibitor, signal transduction protein or its fragment, antibody or its fragment, single chain antibody, binding protein or its fragment, peptide, antigen, adhesive protein, structural protein, regulatory protein, **toxin** protein, cytokine, transcription regulatory protein, blood clotting protein or plant defense-inducing protein;

(7) a genetic carrier library displaying on its surface variants of a protein, prepared by a process comprising the steps (2-a) - (2-e);

(8) bioconversion using protein with **activity** for conversion reaction, characterized in that the **method** employs

the complex between genetic carrier and protein;

(9) producing an antibody to an antigen in vertebrates, characterized in that the **method** comprises administering to vertebrates a composition containing an immunological amount of the complex between genetic carrier and protein; and

(10) a protein microarray comprising a solid substrate and a material immobilized onto the substrate, characterized in that the material immobilized onto the substrate is selected from the complex between genetic carrier and protein; and the genetic carrier library.

USE - The **method** is useful for surface display of proteins, particularly for displaying a protein on the surface of e.g. spores, for improving a protein, or for isolating a substance. These **methods** are useful in obtaining monoclonal variants from a large library, or in high-throughput screening of antibodies for use in therapy (e.g. as vaccines), diagnosis or analysis.

Dwg.0/8

L8 ANSWER 5 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN

ACCESSION NUMBER: 2002-195744 [25] WPIDS
DOC. NO. CPI: C2002-060478
TITLE: Novel synthetic peptides which include cell-growth affecting peptides and peptides which enhance or inhibit cellular protein production, useful for enhancing or inhibiting cell growth or cellular protein production.
DERWENT CLASS: B04 D16
INVENTOR(S): CAMPBELL, R L; ERICKSON, B W; HAALAND, P D; LLOYD, S A; SHERMAN, D B; STEWART, W W
PATENT ASSIGNEE(S): (BECT) BECTON DICKINSON & CO
COUNTRY COUNT: 31
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002002591	A2	20020110	(200225)*	EN	41
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR					
W: AU BR CA CN ES IL JP KR MX NZ RU SG					
AU 2001075173	A	20020114	(200237)		
EP 1317475	A2	20030611	(200339)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002002591	A2	WO 2001-US17943	20010604
AU 2001075173	A	AU 2001-75173	20010604
EP 1317475	A2	EP 2001-941853	20010604
		WO 2001-US17943	20010604

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2001075173	A	Based on	WO 2002002591
EP 1317475	A2	Based on	WO 2002002591

PRIORITY APPLN. INFO: US 2000-608892 20000630

AN 2002-195744 [25] WPIDS

AB WO 200202591 A UPAB: 20020418

NOVELTY - Synthetic peptides (I) which include cell-growth affecting peptides and peptides which enhance or inhibit cellular protein production, selected from 94 peptides given in the specification, are new.

DETAILED DESCRIPTION - (I) is selected from 94 peptides given in the specification such as GEAL, KLAL, EKAL, ESAL, NDAL, NNAL, SNAL, VNAL, KKAL or SKKA.

INDEPENDENT CLAIMS are also included for the following:

(1) a peptide library (II) comprising chemically synthesized peptides, each of the peptides comprising an N-terminal or C-terminal amino acid associated with enzymatic or chemical cleavage of a polypeptide and one or more additional amino acids;

(2) a peptide (III) selected from (II);

(3) a cell or tissue culture medium (IV) comprising (I) or (III); and

(4) production of (III).

ACTIVITY - Antibacterial.

No supporting data provided.

MECHANISM OF ACTION - Inhibitor/enhancer of cellular protein production and/or cellular growth.

No supporting data provided.

USE - (I) or (III) is useful for enhancing or inhibiting cell growth of **Clostridium** perfringens or cellular protein production of beta -**toxin**, by culturing the cells or tissues in the presence of about 0.1-25 mM, preferably 1.0-12 mM of (I) or (III) (claimed). (II) is useful for rapid identification of biologically active compounds which affect the properties of cells in culture. (II) or (III) is useful in concatemer-based recombinant expression **methods** or in large-scale, economical recombinant production **methods**.

ADVANTAGE - (I) reduces the number and quantity of undefined components in culture media, reduces the need for animal-derived components, improves media consistency and quality control, and provides a **method** for precisely controlling and adjusting performance of the cell culture.

Dwg.0/0

L8 ANSWER 6 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2002:168833 BIOSIS

DOCUMENT NUMBER: PREV200200168833

TITLE: In vitro reconstitution of the **Clostridium** botulinum type D progenitor **toxin**.

AUTHOR(S): Kouguchi, Hirokazu; Watanabe, Toshihiro; Sagane, Yoshimasa; Sunagawa, Hiroyuki; Ohyama, Tohru (1)

CORPORATE SOURCE: (1) Department of Food Science and Technology, Faculty of Bioindustry, Tokyo University of Agriculture, 196 Yasaka, Abashiri, 099-2493: t-oyama@bioindustry.nodai.ac.jp Japan

SOURCE: Journal of Biological Chemistry, (January 25, 2002) Vol. 277, No. 4, pp. 2650-2656. http://www.jbc.org/. print. ISSN: 0021-9258.

DOCUMENT TYPE: Article

LANGUAGE: English

AB **Clostridium** botulinum type D strain 4947 produces two different sizes of progenitor **toxins** (M and L) as intact forms without proteolytic processing. The M **toxin** is composed of neurotoxin (NT) and nontoxic-nonhemagglutinin (NTNHA), whereas the L **toxin** is composed of the M **toxin** and hemagglutinin (HA) subcomponents (HA-70, HA-17, and HA-33). The HA-70 subcomponent and the HA-33/17 complex were isolated from the L **toxin** to near homogeneity by chromatography in the presence of denaturing agents. We were able to demonstrate, for the first time, in vitro reconstitution of the L **toxin** formed by mixing purified M **toxin**, HA-70, and HA-33/17. The properties of reconstituted and native L **toxins** are indistinguishable with respect to their gel filtration profiles, native-PAGE profiles, hemagglutination **activity**, binding **activity** to erythrocytes, and oral toxicity to mice. M **toxin**, which contained nicked NTNHA prepared by treatment with trypsin, could no longer be reconstituted to the L **toxin** with HA

subcomponents, whereas the L **toxin** treated with **proteases** was not degraded into M **toxin** and HA subcomponents. We conclude that the M **toxin** forms first by assembly of NT with NTNHA and is subsequently converted to the L **toxin** by assembly with HA-70 and H-33/17.

L8 ANSWER 7 OF 27 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 2003:277358 CAPLUS
TITLE: In vitro determination of specific toxicity in tetanus vaccines
AUTHOR(S): Kegel, B.; Bonifas, U.; Silberbach, K.; Kramer, B.; Weisser, K.
CORPORATE SOURCE: Federal Agency for Sera and Vaccines, Paul-Ehrlich-Institute, Langen, Germany
SOURCE: Developments in Biologicals (Basel, Switzerland) (2002), 111(Advancing Science and Elimination of the Use of Laboratory Animals for Development and Control of Vaccines and Hormones), 27-33
CODEN: DBEIAI; ISSN: 1424-6074
PUBLISHER: S. Karger AG
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Tetanus vaccine is prepd. from detoxified tetanus neurotoxin. To ensure the absence of residual **toxin activity** or to exclude the reversion to toxicity reliable control testing is based on in vivo **methods**, because no in vitro assay provides the required specificity and sensitivity. Tetanus neurotoxin is a 150 kDa protein produced by **Clostridium** tetani. The 50 kDa light chain of this neurotoxin belongs to the family of zinc metalloproteases. It cleaves synaptobrevin, a small synaptic vesicle protein, which is involved in neuroexocytosis, at the single Q76-F77 peptide bond. To develop a sensitive in vitro assay capable of quantifying the proteolytic **activity** of this **toxin**, we used as substrate a recombinant fragment of synaptobrevin2 (1-97). For detecting the cleavage products a peptide antibody raised against the N-terminal cleavage site was used. In Western Blot anal. only the cleaved substrate was detected while the uncleaved substrate showed no signal. In different approaches, recombinant synaptobrevin was either. (i) bound to a microtitre plate, reduced **toxin** was added and the N-terminal cleavage product was detected by a specific antibody or. (ii) the cleavage was performed in test tubes, the samples were transferred to a microtitre plate and immobilized cleavage products were detected. When toxoid or crude **toxin** is used, non-specific cleavage of synaptobrevin substrate occurs. Depending on the toxoid used different patterns of degrdn. of substrate are visible in Western Blots. Different **protease** inhibitors and reaction conditions seem to have an effect on the inhibition of this non-specific cleavage.
REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 8 OF 27 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:152855 CAPLUS
DOCUMENT NUMBER: 134:203683
TITLE: Recombinant construction and expression of single-chain activatable neurotoxins
INVENTOR(S): Dolly, J. Oliver; Li, Yan; Chan, Kuo Chion
PATENT ASSIGNEE(S): Allergan Sales, Inc., USA
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001014570	A1	20010301	WO 2000-US23427	20000825
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
BR 2000012759	A	20020402	BR 2000-12759	20000825
EP 1206554	A1	20020522	EP 2000-964920	20000825
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
JP 2003507073	T2	20030225	JP 2001-518882	20000825

PRIORITY APPLN. INFO.: US 1999-150710P P 19990825
WO 2000-US23427 W 20000825

AB Compns. comprising activable recombinant neurotoxins and polypeptides derived therefrom. The invention also comprises nucleic acids encoding such polypeptides, and **methods** of making such polypeptides and nucleic acids. Thus, a single-chain protein is constructed by genetic engineering techniques comprising the functional domains of a clostridial neurotoxin H chain and some or all of the functions of a clostridial neurotoxin L chain, and having an inserted proteolytic cleavage site located between the H domain and the L domain by which the single-chain protein may be cleaved to produce the individual chains, prefereably covalently linked by a disulfide linkage. To minimize the safety risk assocd. with handling neurotoxins, they are expressed as their low **activity** (or inactive) single-chian proforms, and then carefully activated via cleavage at a site designed to have a high degree of specificity to proteolytic enzymes which do not normally occur in humans. The interchain loop region of the **Clostridium** botulinum subtype E neurotoxin, which is normally resistant to proteolytic nicking in the bacterium and mammals, is modified to include the inserted proteolytic cleavage site. Single-chain tetanus **toxins** contg. a bovine enterokinase cleavage site are expressed from Escherichia coli and shown to induce in vitro paralysis using the mouse phrenic nerve hemi-diaphragm assay. Further modification of single-chain tetanus **toxin** to remove proteolytic cleavage sites reduces the toxicity of unnicked recombinant **toxin**. Single-chain botulin type A and E neurotoxins are also described.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 9 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:283768 BIOSIS
DOCUMENT NUMBER: PREV200100283768
TITLE: High-throughput fluorogenic assay for determination of botulinum type B neurotoxin **protease activity**.
AUTHOR(S): Anne, Christine; Cornille, Fabrice; Lenoir, Christine; Roques, Bernard P. (1)
CORPORATE SOURCE: (1) Departement de Pharmacochimie Moleculaire et Structurale, U266 INSERM, UMR 8600 CNRS, UFR des Sciences Pharmaceutiques et Biologiques, 4, Avenue de l'Observatoire, 75270, Paris Cedex 06: roques@pharmacie.univ-paris5.fr France
SOURCE: Analytical Biochemistry, (April 15, 2001) Vol. 291, No. 2,

pp. 253-261. print.
ISSN: 0003-2697.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Botulinum neurotoxins are responsible for botulism, a flaccid muscular paralysis caused by inhibition of acetylcholine release at the neuromuscular junction. This occurs by cleavage of conserved proteins involved in exocytosis such as synaptobrevin by the zinc metallopeptidase **activity** of the light chain of some botulinum neurotoxins. Botulism, for which there is presently no therapy available, is a relatively widespread disease that may result in death. Consequently, the development of drugs able to inhibit the hydrolytic **activity** of these neurotoxins is of great interest. Design and screening of such inhibitors could be largely facilitated by using high-throughput assays. With this aim, a novel in vitro test for quantifying the proteolytic **activity** of botulinum type B neurotoxin was developed. The substrate is the 60-94 fragment of human synaptobrevin-1 which was modified by introduction of the fluorescent amino acid L-pyrenylalanine in position 74 and a p-nitrophenylalanyl residue as quenching group in position 77. The cleavage of Syb 60-94 (Pya74, Nop77) by the **toxin** active chain occurs selectively between residues 76 and 77 as in the case of the unmodified synaptobrevin and is directly quantified by measuring the strong fluorescence of the formed metabolite Syb 60-76 (Pya74). This is the easiest, quickest, and cheapest assay described to date for measuring the proteolytic **activity** of botulinum type B neurotoxin. It can be easily automated for high-throughput screening. Moreover, amounts of about 3.5 pg/ml of botulinum type B neurotoxin could be detected by this **method**.

L8 ANSWER 10 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:111428 BIOSIS
DOCUMENT NUMBER: PREV200000111428
TITLE: Rescue of exocytosis in botulinum **toxin** A-poisoned chromaffin cells by expression of cleavage-resistant SNAP-25: Identification of the minimal essential C-terminal residues.
AUTHOR(S): O'Sullivan, Gregory A.; Mohammed, Nadiem; Foran, Patrick G.; Lawrence, Gary W.; Dolly, J. Oliver (1)
CORPORATE SOURCE: (1) Department of Biochemistry, Imperial College, London, SW7 2AY UK
SOURCE: Journal of Biological Chemistry, (Dec. 24, 1999) Vol. 274, No. 52, pp. 36897-36904.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Botulinum neurotoxin (BoNT) types A and B selectively block exocytosis by cleavage of SNAP-25 and synaptobrevin, respectively; in humans, many months are required for full recovery from the resultant neuromuscular paralysis. To decipher the molecular basis for such prolonged poisoning, intoxication in adreno-chromaffin cells was monitored over 2 months. Exocytosis from BoNT/B-treated cells resumed after 56 days because of the appearance of intact synaptobrevin. However, inhibition continued in BoNT/A-treated cells, throughout the same interval, with a continued predominance of cleaved SNAP-25-(1-197) over the intact protein. When recovery from poisoning was attempted by transfection of the latter cells with the gene encoding full-length SNAP-25-(1-206), no restoration of exocytosis ensued even after 3 weeks. To ascertain if this failure was because of the persistence of the **toxin**'s **protease activity**, the cells were transfected with BoNT/A-resistant SNAP-25 constructs; importantly, exocytosis was rescued. C-terminal truncation of

the **toxin**-insensitive SNAP-25 revealed that residues 1-201, 1-202, 1-203 afforded a significant return of exocytosis, unlike shorter forms 1-197, -198, -199, or -200; accordingly, mutants M202A or L203A of full-length SNAP-25 rescued secretion. These findings give insights into the C-terminal functional domain of SNAP-25, demonstrate the longevity of BoNT/A **protease**, and provide the prospect of a therapy for botulism.

L8 ANSWER 11 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:186708 BIOSIS
DOCUMENT NUMBER: PREV199900186708
TITLE: Molecular properties of a hemagglutinin purified from type a **Clostridium** botulinum.
AUTHOR(S): Sharma, Shashi Kant; Fu, Fen-Ni; Singh, Bal Ram (1)
CORPORATE SOURCE: (1) Department of Chemistry and Biochemistry, University of Massachusetts Dartmouth, North Dartmouth, MA, 02747 USA
SOURCE: Journal of Protein Chemistry, (Jan., 1999) Vol. 18, No. 1, pp. 29-38.
ISSN: 0277-8033.
DOCUMENT TYPE: Article
LANGUAGE: English
AB **Clostridium** botulinum causes the food poisoning disease botulism by producing botulinum neurotoxin, the most potent **toxin** known. The neurotoxin is produced along with a group of neurotoxin-associated proteins, or NAPs, which protect it from the low pH and **proteases** of the gastrointestinal tract. Recently, we isolated one of the major components of NAPs, a 33-kDa hemagglutinin (Hn-33) (Fu et al. (1998), J. Protein Chem. 17, 53-60). In this study, we present molecular properties of Hn-33 derived from several biochemical and biophysical techniques. Hn-33 in pure form requires a 66-fold lower concentration of sugar inhibition of its hemagglutination **activity** than in its complexed form with the neurotoxin and other NAPs. However, its **protease** resistance is not affected by sugar binding. Based on FT-IR and circular dichroism (CD) analysis, Hn-33 is a predominantly beta-sheet protein (74-77%). Hn-33 analysis by laser desorption mass spectrometry and size exclusion column chromatography reveals that it exists predominantly in a dimeric form in the aqueous solution. Even a very low concentration of SDS (0.05%) irreversibly destroyed the biological **activity** of Hn-33 by changing its secondary structure as revealed by far-UV CD analysis.

L8 ANSWER 12 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1997:440312 BIOSIS
DOCUMENT NUMBER: PREV199799739515
TITLE: The propeptide of **Clostridium** septicum alpha **toxin** functions as an intramolecular chaperone and is a potent inhibitor of alpha **toxin**-dependent cytolysis.
AUTHOR(S): Sellman, Bret R.; Tweten, Rodney K. (1)
CORPORATE SOURCE: (1) Microbiol. Immunol., Univ. Oklahoma Health Sci. Cent., Oklahoma City, OK 73190 USA
SOURCE: Molecular Microbiology, (1997) Vol. 25, No. 3, pp. 429-440.
ISSN: 0950-382X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB **Clostridium** septicum alpha **toxin** is activated by a proteolytic cleavage at Arg-398 in its carboxy terminus, which yields a 41.3-kDa cytolytically active **toxin** and a 5.1-kDa propeptide. Studies were performed to determine when the propeptide dissociated from the **toxin** after proteolytic activation of the protoxin (AT-pro) and to demonstrate the chaperone **activity** of the propeptide. The propeptide was found to remain associated with the **toxin** after

activation with trypsin (AT-act) when analysed by gel filtration or affinity chromatography of a polyhistidine-tagged derivative that contained the polyhistidine tag on the propeptide. The affinity of the propeptide for the **toxin** was decreased significantly when a mutation was introduced in which Val-400 was converted to a cysteine residue. This mutation destabilized the interaction of the propeptide with the **toxin** and the propeptide was found to dissociate from the **toxin** under the same gel-filtration conditions used for the wild-type **toxin**. The separation of the propeptide in the V400C mutant did not affect the cytolytic **activity** of the **toxin** and therefore the propeptide was not necessary for cytolytic **activity**. These data suggested that the propeptide did not dissociate from the main body of the **toxin** after proteolysis. Further analysis demonstrated that purified propeptide was a potent inhibitor of alpha **toxin activity**, which inhibited the oligomerization of alpha **toxin** into a functional pore. These data suggest that the propeptide does not participate in the final oligomerized complex and that oligomerization appears to displace the propeptide from AT-act. The importance of the propeptide to the solution stability of alpha **toxin** was also demonstrated. When AT-pro was activated in solution with trypsin a significant level (approximately 50%) of inactive aggregate formed. This aggregate, which could be removed by centrifugation at 14 000 times g, was made up of both SDS-sensitive and -resistant aggregates, suggesting that a variety of inactive aggregates formed when the monomers interacted in solution. Significantly higher levels of haemolytic **activity** (approximately 16-fold) were observed when alpha **toxin** was proteolytically activated after membrane binding instead of in solution. These results support the role of the propeptide as an intramolecular chaperone that stabilizes the monomeric AT-pro and shuttles it to the membrane where it is activated by **protease**, oligomerizes into a pre-pore complex and forms a pore. The data suggest that oligomerization of the **toxin** displaces the propeptide from the monomer form of alpha **toxin** and that the propeptide does not participate in, and is not necessary to, the final cytolytic complex.

L8 ANSWER 13 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1997:410119 BIOSIS
DOCUMENT NUMBER: PREV199799702162
TITLE: A biotinylated perfringolysin O derivative: A new probe for detection of cell surface cholesterol.
AUTHOR(S): Iwamoto, Machiko (1); Morita, Ikuo; Fukuda, Mitsugu; Murota, Sei-Itsu; Ando, Susumu; Ohno-Iwashita, Yoshiko
CORPORATE SOURCE: (1) Dep. Membrane Biochem., Tokyo Metropolitan Inst. Gerontol., 35-2 Sakae-cho, Itabashi-ku, Tokyo 173 Japan
SOURCE: Biochimica et Biophysica Acta, (1997) Vol. 1327, No. 2, pp. 222-230.
ISSN: 0006-3002.
DOCUMENT TYPE: Article
LANGUAGE: English
AB theta-**Toxin** is a cholesterol-binding, pore-forming cytolysin of **Clostridium** perfringens. To detect cell surface cholesterol, we prepared a theta-**toxin** derivative, BC-theta by biotinylation of a **protease**-nicked theta-**toxin**, which has the same binding affinity for cholesterol as theta-**toxin** without cytolytic **activity**. Human erythrocytes, V79 cells and human umbilical vein endothelial cells (HUVEC), were stained with BCO coupled with FITC-avidin, and then the cells were analyzed by either flow cytometry or laser confocal microscopy. The fluorescence intensity increased in both intact and briefly fixed cells when treated with BC-theta. BC-theta-treated V79 cells were stained by neither trypan blue nor propidium iodide, indicating that BC theta stained just the outer

surface of the plasma membrane of vital cells. Treatment of the cells with digitonin, a cholesterol sequestering reagent, decreased the fluorescence intensity to the background level, indicating that BC-theta staining is specific for cholesterol. The fluorescence intensity of erythrocytes pre-permeabilized with a small amount of theta-**toxin** increased more than ten-fold, suggesting higher cholesterol contents in the inner layer of the plasma membrane. When cells were cultured with cholesterol-depleted medium, the fluorescence intensity stained by BC-theta decreased remarkably in V79 cells, but did not change in HUVEC. This indicates that cell surface cholesterol may be provided in different ways with these two cell lines. These results suggest that BC theta can be a useful probe for visualizing cell surface cholesterol and for evaluating the effects of cellular events on the topology and distribution of cholesterol.

L8 ANSWER 14 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1996:569359 BIOSIS
DOCUMENT NUMBER: PREV199799298715
TITLE: Contribution of individual tryptophan residues to the structure and **activity** of THETA-**toxin** (perfringolysin O), a cholesterol-binding cytolysin.
AUTHOR(S): Sekino-Suzuki, Naoko; Nakamura, Megumi; Mitsui, Ken-Ichiro; Ohno-Iwashita, Yoshiko (1)
CORPORATE SOURCE: (1) Dep. Enzyme Biochemistry, Tokyo Metropolitan Inst. Gerontol., Sakae-cho, Itabashi-ku, Tokyo 173 Japan
SOURCE: European Journal of Biochemistry, (1996) Vol. 241, No. 3, pp. 941-947.
ISSN: 0014-2956.
DOCUMENT TYPE: Article
LANGUAGE: English
AB theta-**Toxin** (perfringolysin O), secreted by **Clostridium** perfringens, shares with other known thiol-activated **toxins** a conserved undecapeptide, ECTGLAWEWWR, located in the C-terminal region of the protein and containing the unique cysteine of the molecule. Single and double amino acid substitutions were created in the theta-**toxin** molecule to investigate the role of individual tryptophan residues in the lytic **activity** of theta-**toxin**. Wild-type and mutant theta-**toxins** were overproduced in Escherichia coli by means of a T7 RNA polymerase/promoter system and purified. The relative hemolytic **activities** of four mutant **toxins**, each with a Trp to Phe substitution outside the common Cys-containing region, were more than 60% that of wild-type theta-**toxin**. In contrast, mutant **toxins** with Phe replacements within the Cys-containing region (at Trp436, Trp438 or Trp439) showed significantly reduced hemolytic and erythrocyte-membrane binding **activities**. The largest reduction in binding affinity, more than 100-fold, was observed for Trp438 mutant **toxins**. However, the mutants retain binding specificity for cholesterol and the ability to form are shaped and ring-shaped structures on membranes. These results indicate that the low hemolytic **activities** of these mutant **toxins** can be ascribed, at least in part, to reduced binding **activities**. With respect to **protease** susceptibility and far-ultraviolet circular-dichroism spectra, only the W436 fwdarw F mutant **toxin**, showed any considerable difference from wild-type **toxin** in secondary or higher-order structures, indicating that Trp436 is essential for maintenance of **toxin** structure.

L8 ANSWER 15 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 3
ACCESSION NUMBER: 1996:422530 BIOSIS
DOCUMENT NUMBER: PREV199699153586
TITLE: Nitric oxide inhibits rat intestinal secretion by

Clostridium difficile **Toxin** A but not Vibrio cholerae enterotoxin.

AUTHOR(S): Qiu, Bosheng; Pothoulakis, Charalabos; Nikulasson, Ignazio Castagliuolo Zigfus; Lamont, J. Thomas (1)

CORPORATE SOURCE: (1) Div. Gastroenterol., Beth Israel Hosp., 330 Brookline Ave., Boston, MA 02215 USA

SOURCE: Gastroenterology, (1996) Vol. 111, No. 2, pp. 409-418. ISSN: 0016-5085.

DOCUMENT TYPE: Article

LANGUAGE: English

AB Background & Aims: Intestinal inflammation is associated with increased synthesis of nitric oxide, whereas inhibition of NO synthase (NOS) reduces experimental chronic intestinal inflammation. The aim of this study was to test the effects of NO blockers and donors on acute intestinal inflammation induced by **Clostridium** difficile **toxin** A in rat ileum. **Methods**: Rats received NOS inhibitors or NO donors before measurement of **toxin**-mediated ileal secretion and permeability changes. Mucosal mast cell and neutrophil **activity** were measured by release of rat mast cell **protease** II and myeloperoxidase **activity**, respectively. Results: NOS inhibitors augmented but an NO donor inhibited **toxin** A-mediated ileal secretion and permeability when given before but not after **toxin** administration. Neither an NOS inhibitor nor an NO donor had any effect on cholera **toxin**-mediated secretion. Mast cell degranulation and neutrophil infiltration occurred after injection of **toxin** A or an NOS inhibitor, whereas the NO donor blocked both **toxin** A effects. Conclusions: NOS inhibitors augmented and an NO donor blocked the intestinal effects of **toxin** A but not of cholera **toxin** . NO protects against **toxin** A by inhibition of intestinal mast cells and neutrophils, which are activated by **toxin** A, but not by cholera **toxin**.

L8 ANSWER 16 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 1995:117495 BIOSIS

DOCUMENT NUMBER: PREV199598131795

TITLE: Differences in the **Protease Activities** of Tetanus and Botulinum B **Toxins** Revealed by the Cleavage of Vesicle-Associated Membrane Protein and Various Sized Fragments.

AUTHOR(S): Foran, Patrick; Shone, Clifford C.; Dolly, J. Oliver (1)

CORPORATE SOURCE: (1) Dep. Biochem., Imperial Coll., London SW7 2AY UK

SOURCE: Biochemistry, (1994) Vol. 33, No. 51, pp. 15365-15374. ISSN: 0006-2960.

DOCUMENT TYPE: Article

LANGUAGE: English

AB Botulinum neurotoxin serotype B (BoNT/B) and tetanus **toxin** (TeTx) block neuroexocytosis through selective endoproteolysis of vesicle-associated membrane protein (VAMP). The enzymological properties of both **toxins** were compared for the first time in their cleavage of VAMP and various sized fragments using a sensitive chromatographic assay. The optimal substrate sizes for the zinc-dependent **protease activities** of the light chains of TeTx and BoNT/B were established using synthetic peptides corresponding to the hydrophilic core of VAMP (30-62 amino acids in length). TeTx was found to selectively cleave the largest peptide at a single site, Gln76-Phe77. It exhibited the most demanding specificity, requiring the entire hydrophilic domain (a 62-mer) for notable hydrolysis, whereas BoNT/B efficiently cleaved the much smaller 40-mer. Thus, an unusually long N-terminal sequence of 44 amino acids upstream of the scissile bond is required for the selective hydrolysis of VAMP by TeTx. Using the largest peptide, BoNT/B and TeTx exhibited apprx 50% and 35%, respectively, of the **activities** shown toward intact VAMP, detergent solubilized from

synaptic vesicles. Given the large size of the smallest substrates, it is possible that these neurotoxins recognize and require a three-dimensional structure. Although both **toxins** were inactivated by divalent metal chelators, neither was antagonized by phosphoramidon or ASQFETS (a substrate-related peptide that spans the cleavage site), and TeTx was only feebly inhibited by captopril; also, they were distinguishable in their relative **activities** at different pHs, temperatures, and ionic strengths. These collective findings are important in the design of effective inhibitors for both **toxins**, as well as in raising the possibility that TeTx and BoNT/B interact somewhat differently with VAMP.

L8 ANSWER 17 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1994:345527 BIOSIS
DOCUMENT NUMBER: PREV199497358527
TITLE: A single mutation in the recombinant light chain of tetanus **toxin** abolishes its proteolytic **activity** and removes the toxicity seen after reconstitution with native heavy chain.
AUTHOR(S): Li, Yan; Foran, Patrick; Fairweather, Niel F.; De Paiva, Anton; Weller, Ulrich; Dougan, Gordon; Dolly, J. Oliver (1)
CORPORATE SOURCE: (1) Dep. Biochem., Imperial Coll., London SW7 2AY UK
SOURCE: Biochemistry, (1994) Vol. 33, No. 22, pp. 7014-7020. ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Specific proteolysis by the tetanus **toxin** light chain of a vesicle-associated membrane protein (VAMP) involved in exocytosis is thought to underlie its intracellular blockade of neurotransmitter release. To substantiate this mechanism, recombinant light chain was expressed as a maltose binding protein-light chain fusion product in Escherichia coli. After purification by affinity chromatography and cleavage with factor Xa, the resultant light chain was isolated and its identity confirmed by Western blotting and N-terminal sequencing. It exhibited **activity** similar to that of the native light chain in proteolyzing its target in isolated bovine small synaptic vesicles and in hydrolyzing a 62-residue synthetic polypeptide spanning the cleavage site of the substrate. The importance of Glu-234 in the catalytic **activity** of the light chain, possibly analogous to Glu-143 of thermolysin, was examined using site-directed mutagenesis. Changing Glu-234 to Ala abolished the **protease activity** of the light chain, but its ability to bind the polypeptide substrate was retained. Each recombinant light chain could be reconstituted with the heavy chain of tetanus **toxin**, yielding the same level of disulfide-linked species as the two native chains. Whereas the **toxin** formed with wild-type light chain exhibited appreciable neuromuscular paralysis **activity** and mouse lethality, the equivalent dichain material containing the Ala-234 mutant lacked neurotoxicity in both the in vitro and in vivo assays. Thus, these results demonstrate directly, for the first time, that the lethality of tetanus **toxin** and its inhibition of exocytosis in intact neurons are attributable largely, if not exclusively, to endoprotease **activity**
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L8 ANSWER 18 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 4
ACCESSION NUMBER: 1994:451181 BIOSIS
DOCUMENT NUMBER: PREV199497464181
TITLE: Neuronal involvement in the intestinal effects of **Clostridium** difficile **toxin** A and Vibrio cholerae enterotoxin in rat ileum.
AUTHOR(S): Castagliuolo, Ignazio; Lamont, J. Thomas; Letourneau, Richard; Kelly, Ciaran; O'Keane, J. Connor; Jaffer, Amir;

Theoharides, Theoharis C.; Pothoulakis, Charalabos (1)
CORPORATE SOURCE: (1) Sect. Gastroenterol., Boston Univ. Med. Center, Univ. Hosp., 88 East Newton St., Boston, MA 02118 USA
SOURCE: Gastroenterology, (1994) Vol. 107, No. 3, pp. 657-665. ISSN: 0016-5085.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Background/Aims: Activation of intestinal mast cells and neurons is involved in intestinal inflammation and diarrhea. This study compared the effects of neuronal inhibitors and inhibition of intestinal sensory afferent nerves on the intestinal actions of **Clostridium** difficile **toxin** A, an inflammatory enterotoxin, and cholera **toxin**, a noninflammatory enterotoxin. **Methods**: The effects of lidocaine, hexamethonium, atropine, and long-term pretreatment of capsaicin on fluid secretion, mannitol permeability, myeloperoxidase (MPO) **activity**, and release of rat mast cell **protease** II (RMCPII) were measured in **toxin** A- and cholera **toxin** -exposed loops in vivo. Results: Lidocaine, hexamethonium, and capsaicin, but not atropine, inhibited **toxin** A-mediated secretion and MPO **activity**, but only capsaicin reduced mannitol permeability. Lidocaine, but not capsaicin, reduced secretion and permeability caused by cholera **toxin**. **Toxin** A caused release of RMCPII from rat ileum in vivo and in vitro; this was inhibited by lidocaine or capsaicin, whereas cholera **toxin** had no effect on release of RMCPII. Conclusions: Neuronal mechanisms are important in the in vivo effects of these two enterotoxins. Capsaicin-sensitive sensory afferent neurons and mast cells are involved in the intestinal mechanism of **toxin** A, but not cholera **toxin**.

L8 ANSWER 19 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1994:227250 BIOSIS
DOCUMENT NUMBER: PREV199497240250
TITLE: Antagonism of the intracellular action of botulinum neurotoxin type A with monoclonal antibodies that map to light-chain epitopes.
AUTHOR(S): Cenci Di Bello, Isabelle; Poulain, Bernard; Shone, Clifford C.; Tauc, Ladislav; Dolly, J. Oliver (1)
CORPORATE SOURCE: (1) Dep. Biochem., Imperial Coll. Sci. Technol. and Med., South Kensington, London SW7 2AY UK
SOURCE: European Journal of Biochemistry, (1994) Vol. 219, No. 1-2, pp. 161-169. ISSN: 0014-2956.
DOCUMENT TYPE: Article
LANGUAGE: English

AB mAbs were produced in mice against highly purified, renatured light chain (LC) of botulinum neurotoxin A (BoNT A) that was immobilised on nitrocellulose to avoid the undesirable use of toxoids. Subcutaneous implants of relatively high amounts (up to 10 mu-g each) of LC allowed its slow release into the systemic circulation and, thus, yielded much higher antibody titres against the underivatized antigen than had hitherto been obtained by conventional immunization. Seven stable hybridoma cell lines were established which secrete mAb of IgG, and IgG-2b subclasses reactive specifically with BoNT A and LC, in native and denatured states, without showing any crossreactivity with types B, E, F or tetanus **toxin**. The pronounced reactivities of three mAbs towards refolded LC or intact **toxin**, observed in immunobinding and precipitation assays, relative to that seen in Western blots imply a preference for conformational epitopes. Though mAbs 4, 5 and 7 failed to neutralize the lethality of BoNT in vivo, administration intraneurally of mAb7 prevented the inhibition of transmitter release normally induced by subsequent extracellular administration of BoNT A. Notably, the latter mAb reacted with a synthetic peptide corresponding to amino acids 28-53 in the

N-terminus of the LC, a highly conserved region in Clostridial neurotoxins reported to be essential for maintaining the tertiary structure of the chain. Most importantly, when mAbs 4 or 7 were microinjected inside ganglionic neurons of Aplysia, each reversed, though transiently, the blockade of acetylcholine release by the **toxin**; this novel finding is discussed in relation to the nature of the zinc-dependent **protease activity** of the **toxin**.

L8 ANSWER 20 OF 27 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1993:183439 CAPLUS
DOCUMENT NUMBER: 118:183439
TITLE: Noncytolytic **toxin** conjugates for therapeutics
INVENTOR(S): Morgan, Alton Charles, Jr.; Abrams, Paul G.
PATENT ASSIGNEE(S): Neorx Corp., USA
SOURCE: PCT Int. Appl., 72 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9304191	A1	19930304	WO 1992-US6823	19920813

W: CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE
PRIORITY APPLN. INFO.: US 1991-745158 19910815
AB Conjugates are disclosed which are useful for modifying target cell functions to achieve therapeutic results. Conjugates may include a noncytolytic **toxin** that does not directly inhibit protein synthesis and is capable of operating through an existing cellular metab. signalling mechanism conjugated to a targeting moiety that constitutes a ligand recognized by the target cell receptor involved in that existing signalling mechanism. Alternatively, the conjugates may include a **toxin** domain capable of directly impacting a target cell metabolic process (e.g. catalyzing conversion of ATP to cAMP) or acting on a substrate implicated in such a process (e.g. actin) conjugated with a targeting moiety specific for the target cell population. **Methods** of using the conjugates are also discussed. In mixed lymphocyte reaction studies with cholera holotoxin and cholera **toxin** B oligomer, the domain responsible for inhibition of proliferation was assocd. with the **toxin**'s enzymic **activity** and likely the A1 subunit. Construction of a cholera **toxin** A1/interleukin-2 conjugate for abrogation of transplant rejection is described, as is modification of retargeted **toxin** to reduce immunogenicity.

L8 ANSWER 21 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1993:504499 BIOSIS
DOCUMENT NUMBER: PREV199396128506
TITLE: Interchange of functional domains switches enzyme specificity: Construction of a chimeric pneumococcal-clostridial cell wall lytic enzyme.
AUTHOR(S): Croux, C.; Ronda, C.; Lopez, R.; Garcia, J. L. (1)
CORPORATE SOURCE: (1) Unidad Genet. Bacteriana, Cent. Invest. Biol., Velazquez 144, 28006 Madrid Spain
SOURCE: Molecular Microbiology, (1993) Vol. 9, No. 5, pp. 1019-1025.
ISSN: 0950-382X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Bacterial autolysins are endogenous enzymes that specifically cleave

covalent bonds in the cell wall. These enzymes show both substrate and bond specificities. The former is related to their interaction with the insoluble substrate whereas the latter determine their site of action. The bond specificity allows their classification as muramidases (lysozymes), glucosaminidases, amidases, and endopeptidases. To demonstrate that the autolysin (LYC muramidase) of **Clostridium** acetobutylicum ATCC824 presents a domainal organization, a chimeric gene (c/c) containing the regions coding for the catalytic domain of the LYC muramidase and the choline-binding domain of the pneumococcal phage CPL1 muramidase has been constructed by in vitro recombination of the corresponding gene fragments. This chimeric construction codes for a choline-binding protein (CLC) that has been purified using affinity chromatography on DEAE-cellulose. Several biochemical tests demonstrate that this rearrangement of domains has generated an enzyme with a choline-dependent muramidase **activity** on pneumococcal cell walls. Since the parental LYC muramidase was choline-independent and unable to degrade pneumococcal cell walls, the formation of this active chimeric enzyme by exchanging protein domains between two enzymes that specifically hydrolyse cell walls of bacteria belonging to different genera shows that a switch on substrate specificity has been achieved. The chimeric CLC muramidase behaved as an autolytic enzyme when it was adsorbed onto a live autolysin-defective mutant of Streptococcus pneumoniae. The construction described here provides experimental support for the theory of modular evolution which assumes that novel proteins have evolved by the assembly of preexisting polypeptide units.

L8 ANSWER 22 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1993:504519 BIOSIS
DOCUMENT NUMBER: PREV199396128526
TITLE: Botulinum neurotoxin A selectively cleaves the synaptic protein SNAP-25.
AUTHOR(S): Blasi, Juan; Chapman, Edwin R.; Link, Egenharde; Binz, Thomas; Yamasaki, Shinji; De Camilli, Pietro; Suedhof, Thomas C.; Niemann, Heiner; Jahn, Reinhard
CORPORATE SOURCE: Howard Hughes Med. Inst., Boyer Center Molecular Med., Yale Univ. Med. Sch., P.O. Box 9812, New Haven, CT 06536 USA
SOURCE: Nature (London), (1993) Vol. 365, No. 6442, pp. 160-163. ISSN: 0028-0836.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Neurotransmitter release is potently blocked by a group of structurally related **toxin** proteins produced by **Clostridium** botulinum. Botulinum neurotoxin type B (BoNT/B) and tetanus **toxin** (TeTx) are zinc-dependent **proteases** that specifically cleave synaptobrevin (VAMP), a membrane protein of synaptic vesicles. Here we report that inhibition of transmitter release from synaptosomes caused by botulinum neurotoxin A (BoNT/A) is associated with the selective proteolysis of the synaptic protein SNAP-25. Furthermore, isolated or recombinant L chain of BoNT/A cleaves SNAP-25 in vitro. Cleavage occurred near the carboxyterminus and was sensitive to divalent cation chelators. In addition, a glutamate residue in the BoNT/A L chain, presumably required to stabilize a water molecule in the zinc-containing catalytic centre, was required for proteolytic **activity**. These findings demonstrate that BoNT/A acts as a zinc-dependent **protease** that selectively cleaves SNAP-25. Thus, a second component of the putative fusion complex mediating synaptic vesicle exocytosis is targeted by a clostridial neurotoxin.

L8 ANSWER 23 OF 27 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 92174925 EMBASE
DOCUMENT NUMBER: 1992174925
TITLE: Proteolytic **activity** of **Clostridium** difficile.

AUTHOR: Seddon S.V.; Borriello S.P.
CORPORATE SOURCE: Microb. Pathogenicity Research Group, MRC Clinical Research Centre, Watford Road,Harrow, Middlesex HA1 3UJ, United Kingdom
SOURCE: Journal of Medical Microbiology, (1992) 36/5 (307-311). ISSN: 0022-2615 CODEN: JMMIAV
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Ten isolates of **Clostridium** difficile expressing different degrees of toxigenicity and virulence in an animal model were assayed for the production of proteolytic enzymes by various **methods**. All strains demonstrated some **activity** in one or more of the assay systems. There was no direct correlation between toxigenic status and enzyme production. However, those strains known to be highly virulent in a hamster model were the most proteolytic. The most commonly detected enzyme was cell associated and its substrate specificity suggested it was a trypsin-like enzyme. Initial purification of the enzyme from strain VPI 10463 gave a 10% yield with a 14-fold increase in purity. Inhibition studies on this preparation indicated that the enzyme was a thiol **protease**. The enzyme has pH and temperature optima of 7.5 and 37.degree.C, respectively. These characteristics suggest that the enzyme is more related to clostripain, the thiol clostridio-peptidase of C. histolyticum, than to trypsin. Whilst the role of this enzyme remains unclear, it is possible that it may be a contributory factor in the virulence of the organism as described for other clostridial infections.

L8 ANSWER 24 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 5
ACCESSION NUMBER: 1990:49622 BIOSIS
DOCUMENT NUMBER: BA89:26986
TITLE: INTERRELATIONSHIPS BETWEEN DIGESTIVE PROTEOLYTIC **ACTIVITIES** AND PRODUCTION AND QUANTITATION OF **TOXINS** IN PSEUDOMEMBRANOUS COLITIS INDUCED BY **CLOSTRIDIUM**-DIFFICILE IN GNOTOBIOTIC MICE.
AUTHOR(S): CORTHIER G; MULLER M C; ELMER G W; LUCAS F; DUBOS-RAMARE F
CORPORATE SOURCE: LABORATOIRE D'ECOLOGIE MICROBIENNE, INSTITUT NATL. DE LA RECHERCHE AGRONOMIQUE, CRJ, 78350 JOUY-EN-JOSAS, FRANCE.
SOURCE: INFECT IMMUN, (1989) 57 (12), 3922-3927. CODEN: INFIBR. ISSN: 0019-9567.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB **Clostridium** difficile pathogenicity is related to in vivo production of **toxins**, and it is of great interest to detect **toxins** produced in biological samples. Several reports have shown that **proteases** in stools interfere with immunological **methods** for quantitation of **toxin** A. The purpose of this work was to estimate the relationship between the **proteases** and the C. difficile **toxins** produced in a gnotobiotic mouse model of pseudomembraneous cecitis. Cecal proteolytic **activities** hydrolyzed **toxin** A, and immunoglobulin G bound to the microtiter plate used in immunoassays. This interference could be blocked by the addition of trypsin inhibitor to the samples. The ability of soluble **toxin** A to bind to bound antibodies in an enzyme-linked immunosorbent assay was not affected by the **proteases**, but the biological **activity** was reduced 100-fold. The cytotoxicity of **toxin** B was not modified by proteolytic **activity** treatment. Mice inoculated with a low **toxin** A-producing strain of C. difficile did not die, and no modulation of proteoytic **activities** occurred. After inoculation with the lethal VPI strain

of C. difficle, **toxins** A and B reached maximum levels in the ceca at 12 h postinfection. At this time, the proteolytic **activities** did not decrease from the levels seen at zero time. Mice died within 2 days. At this time (about 32 postinfection), proteolytic **activities** were sharply decreased in the lower parts of the digestive tracts. The findings that serum inhibited the **proteases** and that there was a 100-fold increase in serum-derived mouse immunoglobulins in the lumen as the C. difficle infection progressed suggest that decrease in **protease activity** in the lower digestive tract may be related to the exudation of serum from the inflammation process.

L8 ANSWER 25 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 6
ACCESSION NUMBER: 1988:51196 BIOSIS
DOCUMENT NUMBER: BA85:28055
TITLE: ROLE OF THE ESSENTIAL THIOL GROUP IN THE THIOL-ACTIVATED CYTOLYSIN FROM **CLOSTRIDIUM**-PERFRINGENS.
AUTHOR(S): IWAMOTO M; OHNO-IWASHITA Y; ANDO S
CORPORATE SOURCE: DEP. BIOCHEM., TOKYO METROPOLITAN INST. GERONTOL., 35-2 SAKAECHO, ITABASHI-KU, TOKYO, JPN 173.
SOURCE: EUR J BIOCHEM, (1987) 167 (3), 425-430.
CODEN: EJBCAI. ISSN: 0014-2956.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A hemolysin, .theta.-**toxin**, produced by **Clostridium** perfringens has one cysteinyl residue in the free thiol form which is essential for its hemolytic **activity**. The cysteinyl residue was shown to be located at a position about 5 kDa from the C terminus of the molecule by the **method** of cysteine-specific chemical cleavage. Modification of the residue with a thiol-blocking agent, 5,5'-dithiobis(2-nitrobenzoic acid), reduced the binding affinity of the **toxin** to sheep erythrocytes to 1/100 that of intact **toxin** , resulting in a failure of binding at low cell concentrations (0.5%). Thus the failure of hemolysis at low cell concentrations is primarily ascribed to a decreased affinity of the **toxin** for erythrocytes. Effects of the modification on the lytic processes were examined using high cell concentrations where considerable amounts of modified **toxin** bound to the cells. The modified **toxin** hemolyzes erythrocytes once it binds to them; however, the efficiency of hemolysis is reduced by the modification. These, and additional results indicating that modification alters the sensitivity of **toxin** molecules to **protease** digestion, show that thiol-modification inactivates the **toxin** by affecting both binding and the subsequent lytic processes, probably though a conformational change introduced in the **toxin** molecules.

L8 ANSWER 26 OF 27 MEDLINE on STN
ACCESSION NUMBER: 77005264 MEDLINE
DOCUMENT NUMBER: 77005264 PubMed ID: 965092
TITLE: Molecular forms of neurotoxins in proteolytic **Clostridium** botulinum type B cultures.
AUTHOR: Dasgupta B R; Sugiyama H
SOURCE: INFECTION AND IMMUNITY, (1976 Sep) 14 (3) 680-6.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197612
ENTRY DATE: Entered STN: 19900313
Last Updated on STN: 19970203

Entered Medline: 19761201

AB A modified purification **method** was used to isolate the neurotoxin of proteolytic **Clostridium** botulinum type B strain Lamanna. The preparation was found to be a mixture of two protein forms. They were of molecular weight 152,000 and could not be separated by ion-exchange chromatography or electrophoresis in polyacrylamide gel. One was a single polypeptide chain, and the other was a dichain molecule (nicked **toxin**) held together by an interchain disulfide bond(s). Trypsinization increased the toxicity of the **toxin** preparation and converted the single-chain molecules into dichain forms that were indistinguishable from the endogenously generated nicked **toxin**. A **protease** of the type B culture, with substrate specificity similar to that of trypsin, did not change detectably the molecular form of unnicked type E **toxin**, although toxicity was increased. Higher toxicity was obtained when unnicked type E was trypsinized; the resulting preparation contained only nicked **toxin** molecules.

L8 ANSWER 27 OF 27 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1970:53359 CAPLUS
DOCUMENT NUMBER: 72:53359
TITLE: Dynamics of the **activity** of **toxin** components in microbiol matter and culture broths. I
AUTHOR(S): Shemanova, G. F.; Gorshkova, V. I.; Borisova, O. K.; Shakhanina, K. L.
CORPORATE SOURCE: Inst. Med.-Biol. Probl., Moscow, USSR
SOURCE: Vop. Fiziol. Razmnozheniya Mikroorganizmov Ikh Identifikatsii (1968), 136-50. Editor(s): Tarkov, M. I. Izd. "Kartya Moldovenyaske": Kishinev, USSR. CODEN: 21KZAS
DOCUMENT TYPE: Conference
LANGUAGE: Russian

AB Together with the detn. of basic components of **toxins** (lecithinase, collagenase, **protease**) a **method** was established to det. the **activity** of **toxin** components in culture broth isolated from bacterial suspensions of Clostr idium perfringens. A correlation between the secretion max. of collagenase and lecithinase and the active growing state of microbes which stopped completely in 24-36 hr was obsd. The secretion of alk. **protease** started in 6-9 hr and reached its max. in the 24th hr. In ribosomal fractions and in the hyaloplasm of C. perfringens, alk. **protease** was found but no lecithinase was detected. Small amts. of collagenase were detd. in the 48-72 hr culture. Decreasing of ribosomes is related to the decreasing of the RNA acid-protein ratio. There was no difference in the sedimentation diagrams of ribosomes coming from the 9 hr culture of C. perfringens toxic or nontoxic strain.

=> FIL STNGUIDE

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FULL ESTIMATED COST	92.68	92.89

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CA SUBSCRIBER PRICE	-3.26	-3.26

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                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.24      93.13

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                       ENTRY    SESSION
CA SUBSCRIBER PRICE                                     0.00      -3.26

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L9          38 L6 AND TOXIN

=> s l9 and FRET
L10          0 L9 AND FRET

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L11          0 L9 AND FRET

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L12          0 L9 AND FLUOROPHORE

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L13          0 L9 AND ENERGY

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L14          0 L9 AND STEWARD

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     (FILE 'HOME' ENTERED AT 18:01:49 ON 13 SEP 2003)

     FILE 'BIOSIS, CAPLUS, MEDLINE, WPIDS, EMBASE, SCISEARCH' ENTERED AT
     18:02:04 ON 13 SEP 2003
                E STEWARD
L1           0 S E3 AND LANCE
L2           0 S E3 AND CLOSTRIDIUM
L3           0 S E3 AND PROTEASE
L4        1695 S PROTEASE AND CLOSTRIDIUM
L5         873 S L4 AND ACTIVITY
L6         142 S L5 AND METHOD
L7          38 S L6 AND TOXIN
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L8            27 DUP REM L7 (11 DUPLICATES REMOVED)

      FILE 'STNGUIDE' ENTERED AT 18:06:36 ON 13 SEP 2003

      FILE 'BIOSIS, CAPLUS, MEDLINE, WPIDS, EMBASE, SCISEARCH' ENTERED AT
      18:09:00 ON 13 SEP 2003
L9            38 S L6 AND TOXIN
L10            0 S L9 AND FRET
L11            0 S L9 AND FRET
L12            0 S L9 AND  FLUOROPHORE
L13            0 S L9 AND ENERGY
L14            0 S  L9 AND STEWARD

=> s l6 and fret
L15            0 L6 AND FRET

=> s l6 and fluorescence
L16           11 L6 AND FLUORESCENCE

=> d l16 ibib abs

L16  ANSWER 1 OF 11  BIOSIS  COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER:    2003:113075  BIOSIS
DOCUMENT NUMBER:     PREV200300113075
TITLE:               Fluorigenic substrates for the protease
                     activities of botulinum neurotoxins, serotypes A,
                     B, and F.
AUTHOR(S):           Schmidt, James J. (1); Stafford, Robert G.
CORPORATE SOURCE:    (1) Toxinology and Aerobiology Division, United States Army
                     Medical Research Institute of Infectious Diseases, 1425
                     Porter St., Fort Detrick, MD, 21702-5011, USA:
                     james.schmidt@det.amedd.army.mil USA
SOURCE:              Applied and Environmental Microbiology, (January 2003,
                     2003) Vol. 69, No. 1, pp. 297-303. print.
                     ISSN: 0099-2240.
DOCUMENT TYPE:       Article
LANGUAGE:            English
AB   The seven botulinum neurotoxins (BoNTs) are zinc metalloproteases that
     cleave neuronal proteins involved in neurotransmitter release and are
     among the most toxic natural products known. High-throughput BoNT assays
     are needed for use in antibotulinum drug discovery and to characterize
     BoNT protease activities. Compared to other
     proteases, BoNTs exhibit unusually stringent substrate
     requirements with respect to amino acid sequences and polypeptide lengths.
     Nonetheless, we have devised a strategy for development of fluorigenic
     BoNT protease assays, based on earlier structure-function
     studies, that has proven successful for three of the seven serotypes: A,
     B, and F. In synthetic peptide substrates, the P1 and P3' residues were
     substituted with 2,4-dinitrophenyl-lysine and S-(N-(4-methyl-7-
     dimethylamino-coumarin-3-yl)-carboxamidomethyl)-cysteine, respectively. By
     monitoring the BoNT-catalyzed increase in fluorescence over
     time, initial hydrolysis rates could be obtained in 1 to 2 min when BoNT
     concentrations were 60 ng/ml (about 1 nM) or higher. Each BoNT cleaved its
     fluorigenic substrate at the same location as in the neuronal target
     protein, and kinetic constants indicated that the substrates were
     selective and efficient. The fluorigenic assay for BoNT B was used to
     characterize a new competitive inhibitor of BoNT B protease
     activity with a Ki value of 4 muM. In addition to real-time
     activity measurements, toxin concentration determinations, and
     kinetic studies, the BoNT substrates described herein may be directly
     incorporated into automated high-throughput assay systems to screen large
     numbers of compounds for potential antibotulinum drugs.
```

=> d l16 ibib abs 1-11

L16 ANSWER 1 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:113075 BIOSIS
DOCUMENT NUMBER: PREV200300113075
TITLE: Fluorigenic substrates for the **protease activities** of botulinum neurotoxins, serotypes A, B, and F.
AUTHOR(S): Schmidt, James J. (1); Stafford, Robert G.
CORPORATE SOURCE: (1) Toxinology and Aerobiology Division, United States Army Medical Research Institute of Infectious Diseases, 1425 Porter St., Fort Detrick, MD, 21702-5011, USA: james.schmidt@det.amedd.army.mil USA
SOURCE: Applied and Environmental Microbiology, (January 2003, 2003) Vol. 69, No. 1, pp. 297-303. print.
ISSN: 0099-2240.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The seven botulinum neurotoxins (BoNTs) are zinc metalloproteases that cleave neuronal proteins involved in neurotransmitter release and are among the most toxic natural products known. High-throughput BoNT assays are needed for use in antibotulinum drug discovery and to characterize BoNT **protease activities**. Compared to other **proteases**, BoNTs exhibit unusually stringent substrate requirements with respect to amino acid sequences and polypeptide lengths. Nonetheless, we have devised a strategy for development of fluorigenic BoNT **protease** assays, based on earlier structure-function studies, that has proven successful for three of the seven serotypes: A, B, and F. In synthetic peptide substrates, the P1 and P3' residues were substituted with 2,4-dinitrophenyl-lysine and S-(N-(4-methyl-7-dimethylamino-coumarin-3-yl)-carboxamidomethyl)-cysteine, respectively. By monitoring the BoNT-catalyzed increase in **fluorescence** over time, initial hydrolysis rates could be obtained in 1 to 2 min when BoNT concentrations were 60 ng/ml (about 1 nM) or higher. Each BoNT cleaved its fluorigenic substrate at the same location as in the neuronal target protein, and kinetic constants indicated that the substrates were selective and efficient. The fluorigenic assay for BoNT B was used to characterize a new competitive inhibitor of BoNT B **protease activity** with a Ki value of 4 muM. In addition to real-time **activity** measurements, toxin concentration determinations, and kinetic studies, the BoNT substrates described herein may be directly incorporated into automated high-throughput assay systems to screen large numbers of compounds for potential antibotulinum drugs.

L16 ANSWER 2 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:247143 BIOSIS
DOCUMENT NUMBER: PREV200200247143
TITLE: Rapid determination of substrate specificity of **Clostridium** histolyticum beta-collagenase using an immobilized peptide library.
AUTHOR(S): Hu, Yongbo; Webb, Erin; Singh, Jasbir; Morgan, Barry A.; Gainor, James A.; Gordon, Thomas D.; Siahaan, Teruna J. (1)
CORPORATE SOURCE: (1) Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, 66045: siahaan@ku.edu USA
SOURCE: Journal of Biological Chemistry, (March 8, 2002) Vol. 277, No. 10, pp. 8366-8371. http://www.jbc.org/. print.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The molecular basis of the substrate specificity of **Clostridium**

histolyticum beta-collagenase was investigated using a combinatorial **method**. An immobilized positional peptide library, which contains 24,000 sequences, was constructed with a 7-hydroxycoumarin-4-propanoyl (Cop) fluorescent group attached at the N terminus of each sequence. This immobilized peptide library was incubated with C. histolyticum beta-collagenase, releasing fluorogenic fragments in the solution phase. The relative substrate specificity (kcat/Km) for each member of the library was determined by measuring **fluorescence** intensity in the solution phase. Edman sequencing was used to assign structure to subsites of active substrate mixtures. Collectively, the substrate preference for subsites (P3-P4') of C. histolyticum beta-collagenase was determined. The last position on the C-terminal side in which the identity of the amino acids affects the **activity** of the enzyme is P4', and an aromatic side chain is preferred in this position. The optimal P1'-P3' extended substrate sequence is P1'-Gly/Ala, P2'-Pro/Xaa, and P3'-Lys/Arg/Pro/Thr/Ser. The Cop group in either the P2 or P3 position is required for a high substrate **activity** with C. histolyticum beta-collagenase. S2 and S3 sites of the **protease** play a dominant role in fixing the substrate specificity. The immobilized peptide library proved to be a powerful approach for assessing the substrate specificity of C. histolyticum beta-collagenase, so it may be applied to the study of other **proteases** of interest.

L16 ANSWER 3 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:283768 BIOSIS
DOCUMENT NUMBER: PREV200100283768
TITLE: High-throughput fluorogenic assay for determination of botulinum type B neurotoxin **protease activity**.
AUTHOR(S): Anne, Christine; Cornille, Fabrice; Lenoir, Christine; Roques, Bernard P. (1)
CORPORATE SOURCE: (1) Departement de Pharmacochimie Moleculaire et Structurale, U266 INSERM, UMR 8600 CNRS, UFR des Sciences Pharmaceutiques et Biologiques, 4, Avenue de l'Observatoire, 75270, Paris Cedex 06: roques@pharmacie.univ-paris5.fr France
SOURCE: Analytical Biochemistry, (April 15, 2001) Vol. 291, No. 2, pp. 253-261. print.
ISSN: 0003-2697.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Botulinum neurotoxins are responsible for botulism, a flaccid muscular paralysis caused by inhibition of acetylcholine release at the neuromuscular junction. This occurs by cleavage of conserved proteins involved in exocytosis such as synaptobrevin by the zinc metallopeptidase **activity** of the light chain of some botulinum neurotoxins. Botulism, for which there is presently no therapy available, is a relatively widespread disease that may result in death. Consequently, the development of drugs able to inhibit the hydrolytic **activity** of these neurotoxins is of great interest. Design and screening of such inhibitors could be largely facilitated by using high-throughput assays. With this aim, a novel in vitro test for quantifying the proteolytic **activity** of botulinum type B neurotoxin was developed. The substrate is the 60-94 fragment of human synaptobrevin-1 which was modified by introduction of the fluorescent amino acid L-pyrenylalanine in position 74 and a p-nitrophenylalanyl residue as quenching group in position 77. The cleavage of Syb 60-94 (Pya74, Nop77) by the toxin active chain occurs selectively between residues 76 and 77 as in the case of the unmodified synaptobrevin and is directly quantified by measuring the strong **fluorescence** of the formed metabolite Syb 60-76 (Pya74). This is the easiest, quickest, and cheapest assay described to date for

measuring the proteolytic **activity** of botulinum type B neurotoxin. It can be easily automated for high-throughput screening. Moreover, amounts of about 3.5 pg/ml of botulinum type B neurotoxin could be detected by this **method**.

L16 ANSWER 4 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1997:410119 BIOSIS
DOCUMENT NUMBER: PREV199799702162
TITLE: A biotinylated perfringolysin O derivative: A new probe for detection of cell surface cholesterol.
AUTHOR(S): Iwamoto, Machiko (1); Morita, Ikuo; Fukuda, Mitsugu; Murota, Sei-Itsu; Ando, Susumu; Ohno-Iwashita, Yoshiko
CORPORATE SOURCE: (1) Dep. Membrane Biochem., Tokyo Metropolitan Inst. Gerontol., 35-2 Sakae-cho, Itabashi-ku, Tokyo 173 Japan
SOURCE: Biochimica et Biophysica Acta, (1997) Vol. 1327, No. 2, pp. 222-230.
ISSN: 0006-3002.
DOCUMENT TYPE: Article
LANGUAGE: English
AB theta-Toxin is a cholesterol-binding, pore-forming cytolysin of **Clostridium** perfringens. To detect cell surface cholesterol, we prepared a theta-toxin derivative, BC-theta by biotinylation of a **protease**-nicked theta-toxin, which has the same binding affinity for cholesterol as theta-toxin without cytolytic **activity**. Human erythrocytes, V79 cells and human umbilical vein endothelial cells (HUVEC), were stained with BCO coupled with FITC-avidin, and then the cells were analyzed by either flow cytometry or laser confocal microscopy. The **fluorescence** intensity increased in both intact and briefly fixed cells when treated with BC-theta. BC-theta-treated V79 cells were stained by neither trypan blue nor propidium iodide, indicating that BC theta stained just the outer surface of the plasma membrane of vital cells. Treatment of the cells with digitonin, a cholesterol sequestering reagent, decreased the **fluorescence** intensity to the background level, indicating that BC-theta staining is specific for cholesterol. The **fluorescence** intensity of erythrocytes pre-permeabilized with a small amount of theta-toxin increased more than ten-fold, suggesting higher cholesterol contents in the inner layer of the plasma membrane. When cells were cultured with cholesterol-depleted medium, the **fluorescence** intensity stained by BC-theta decreased remarkably in V79 cells, but did not change in HUVEC. This indicates that cell surface cholesterol may be provided in different ways with these two cell lines. These results suggest that BC theta can be a useful probe for visualizing cell surface cholesterol and for evaluating the effects of cellular events on the topology and distribution of cholesterol.

L16 ANSWER 5 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1995:251557 BIOSIS
DOCUMENT NUMBER: PREV199598265857
TITLE: Structural studies on the zinc-endopeptidase light chain of tetanus neurotoxin.
AUTHOR(S): De Filippis, Vincenzo (1); Vangelista, Luca; Schiavo, Giampietro; Tonello, Fiorella; Montecucco, Cesare
CORPORATE SOURCE: (1) Centro Ricerca Interdipartimentale Biotechnol. Innovative, Via Trieste 75, I-35121 Padova Italy
SOURCE: European Journal of Biochemistry, (1995) Vol. 229, No. 1, pp. 61-69.
ISSN: 0014-2956.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Tetanus neurotoxin (TeNT) blocks neuroexocytosis via a zinc-endopeptidase **activity** highly specific for vescicle-associated membrane protein(VAMP)/synaptobrevin. TeNT is the prototype of clostridial

neurotoxins, a new family of metalloproteinases. They consist of three domains and the proteolytic **activity** is displayed by the 50-kDa light chain (L chain). The L chain was isolated here in the native state from bacterial filtrates of **Clostridium** tetani and its structure was studied via circular dichroism (CD) and **fluorescence** spectroscopy. The secondary structure content (27% alpha-helix and 43% beta-sheet), estimated by far-ultraviolet CD measurements, was in reasonable agreement with that obtained by standard predictive **methods** (25% alpha-helix and 49% beta-sheet). Moreover. the hypothetical zinc-binding motif, encompassing residues His-Glu-Leu-Ile-His, was correctly predicted to be in alpha-helical conformation, as also expected on the basis of the geometrical requirements for a correct coordination of the zinc ion. Both near-ultraviolet CD and **fluorescence** data strongly suggest that the single Trp43 residue is buried and constrained in a hydrophobic environment, likely distant from the zinc ion located in the active-site cleft. The contribution of the bound zinc ion to the overall conformation of TeNT L chain was investigated by different and complementary techniques, including spectroscopic (far- and near-ultraviolet CD, **fluorescence**, second derivative absorption spectroscopy) as well as proteolytic probes. The results indicate that the zinc ion plays little, if any, role in determining the structural properties of the L chain molecule. Similarly, the metal-free apo-enzyme and the holo-protein share common stability features evaluated in respect to different physico-chemical parameters (pH, temperature and urea concentration). These results parallel those obtained on thermolysin, a zinc-dependent neutral endoprotease from Bacillus thermoproteolyticus, where both conformational and stability properties are unchanged upon zinc removal.

L16 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:218561 CAPLUS
DOCUMENT NUMBER: 137:29736
TITLE: Rapid determination of substrate specificity of **Clostridium** histolyticum .beta.-collagenase using an immobilized peptide library
AUTHOR(S): Hu, Yongbo; Webb, Erin; Singh, Jasbir; Morgan, Barry A.; Gainor, James A.; Gordon, Thomas D.; Siahaan, Teruna J.
CORPORATE SOURCE: Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS, 66045, USA
SOURCE: Journal of Biological Chemistry (2002), 277(10), 8366-8371
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The mol. basis of the substrate specificity of **Clostridium** histolyticum .beta.-collagenase was investigated using a combinatorial **method**. An immobilized positional peptide library, which contains 24,000 sequences, was constructed with a 7-hydroxycoumarin-4-propanoyl (Cop) fluorescent group attached at the N terminus of each sequence. This immobilized peptide library was incubated with C. histolyticum .beta.-collagenase, releasing fluorogenic fragments in the soln. phase. The relative substrate specificity (kcat/Km) for each member of the library was detd. by measuring **fluorescence** intensity in the soln. phase. Edman sequencing was used to assign structure to subsites of active substrate mixts. Collectively, the substrate preference for subsites (P3-P4') of C. histolyticum .beta.-collagenase was detd. The last position on the C-terminal side in which the identity of the amino acids affects the **activity** of the enzyme is P4', and an arom. side chain is preferred in this position. The optimal P1'-P3' extended

substrate sequence is P1'-Gly/Ala, P2'-Pro/Xaa, and P3'-Lys/Arg/Pro/Thr/Ser. The Cop group in either the P2 or P3 position is required for a high substrate **activity** with C. histolyticum .beta.-collagenase. S2 and S3 sites of the **protease** play a dominant role in fixing the substrate specificity. The immobilized peptide library proved to be a powerful approach for assessing the substrate specificity of C. histolyticum .beta.-collagenase, so it may be applied to the study of other **proteases** of interest.

REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 7 OF 11 MEDLINE on STN
ACCESSION NUMBER: 2002139165 MEDLINE
DOCUMENT NUMBER: 21864141 PubMed ID: 11724807
TITLE: Rapid determination of substrate specificity of **Clostridium** histolyticum beta-collagenase using an immobilized peptide library.
AUTHOR: Hu Yongbo; Webb Erin; Singh Jasbir; Morgan Barry A; Gainor James A; Gordon Thomas D; Siahaan Teruna J
CORPORATE SOURCE: Department of Pharmaceutical Chemistry, the University of Kansas, Lawrence, Kansas 66045, USA.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 Mar 8) 277 (10) 8366-71.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200204
ENTRY DATE: Entered STN: 20020305
Last Updated on STN: 20030105
Entered Medline: 20020415

AB The molecular basis of the substrate specificity of **Clostridium** histolyticum beta-collagenase was investigated using a combinatorial **method**. An immobilized positional peptide library, which contains 24,000 sequences, was constructed with a 7-hydroxycoumarin-4-propanoyl (Cop) fluorescent group attached at the N terminus of each sequence. This immobilized peptide library was incubated with C. histolyticum beta-collagenase, releasing fluorogenic fragments in the solution phase. The relative substrate specificity (k(cat)/K(m)) for each member of the library was determined by measuring **fluorescence** intensity in the solution phase. Edman sequencing was used to assign structure to subsites of active substrate mixtures. Collectively, the substrate preference for subsites (P(3)-P(4)') of C. histolyticum beta-collagenase was determined. The last position on the C-terminal side in which the identity of the amino acids affects the **activity** of the enzyme is P(4)', and an aromatic side chain is preferred in this position. The optimal P(1)'-P(3)' extended substrate sequence is P(1)'-Gly/Ala, P(2)'-Pro/Xaa, and P(3)'-Lys/Arg/Pro/Thr/Ser. The Cop group in either the P(2) or P(3) position is required for a high substrate **activity** with C. histolyticum beta-collagenase. S(2) and S(3) sites of the **protease** play a dominant role in fixing the substrate specificity. The immobilized peptide library proved to be a powerful approach for assessing the substrate specificity of C. histolyticum beta-collagenase, so it may be applied to the study of other **proteases** of interest.

L16 ANSWER 8 OF 11 MEDLINE on STN
ACCESSION NUMBER: 96373022 MEDLINE
DOCUMENT NUMBER: 96373022 PubMed ID: 8776761
TITLE: Expression and purification of a recombinant "small" sialidase from **Clostridium** perfringens A99.

AUTHOR: Kruse S; Kleineidam R G; Roggentin P; Schauer R
CORPORATE SOURCE: Biochemisches Institut, Christian-Albrechts-Universitat, Kiel, Germany.
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (1996 Jun) 7 (4) 415-22.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 20000303
Entered Medline: 19961205

AB A 1.4-kb gene encoding the "small" sialidase isoenzyme of **Clostridium** perfringens A99, including its own promoter, was previously cloned in and expressed by Escherichia coli JM 101. Since all attempts to purify this enzyme to homogeneity were unsuccessful, a new strategy was developed. The structural gene was amplified by means of a PCR technique and inserted into the plasmid vector pQE-10, transferring a six-histidine affinity tag (His6) to the N-terminus of the protein. In order to minimize proteolytic degradation of the sialidase protein, the gene was subcloned into the Escherichia coli strain BL21(DE3)pLys S with reduced **protease activity**. The sialidase production was increased about 2.5-fold when compared with that of the original clone. The enzyme, released by lysozyme treatment of the bacterial cells, was purified by metal chelate chromatography on Ni-nitrilo-triacetic acid agarose to apparent homogeneity in SDS-PAGE. The 42-kDa protein was enriched 62-fold with a yield of 82% and a specific **activity** of 280 U mg-1. A total amount of 1 mg sialidase was obtained from 1 liter of bacterial culture. For future studies, including crystallization experiments, the histidine affinity tag was removed from the sialidase enzyme by aminopeptidase K. The sialidase was then separated from aminopeptidase K by ion-exchange chromatography, resulting in an overall yield of 83% and a specific **activity** of 305 U mg-1 using 4-methylumbelliferyl- alpha-D-N-acetylneuraminic acid under standard conditions. The two forms (with or without the histidine tag) of sialidase exhibited similar kinetic properties when compared to the wild-type enzyme.

L16 ANSWER 9 OF 11 MEDLINE on STN
ACCESSION NUMBER: 94071072 MEDLINE
DOCUMENT NUMBER: 94071072 PubMed ID: 8250226
TITLE: Synthesis of N alpha-[3H]acetyl-L-lysine chloromethyl ketone and its use in the fluorographic detection of **proteases**.
AUTHOR: Nishikata M
CORPORATE SOURCE: Central Research Division, School of Dentistry, Hokkaido University, Sapporo, Japan.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1993 Oct) 214 (1) 222-6.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940201
Last Updated on STN: 20000303
Entered Medline: 19940105

AB Tritiated N alpha-acetyl-L-lysine chloromethyl ketone (ALCK) was synthesized on a laboratory scale for use as an active-site-directed affinity label in the fluorographic detection of **proteases** after

sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The synthesis involved acetylation of N epsilon-benzyloxycarbonyl-L-lysine chloromethyl ketone with [3H]acetic anhydride just before the removal of the benzyloxycarbonyl group. By this **method**, [3H]ALCK with a specific **activity** of 250 mCi/mmol was obtained as a crystal. Trypsin, thrombin, plasmin, papain, and clostripain were inactivated by ALCK according to first-order kinetics. For fluorographic detection of **proteases**, enzyme samples were allowed to react with [3H]ALCK and then resolved by SDS-PAGE. **Proteases** that reacted with [3H]ALCK could be detected with a sensitivity equivalent to or higher than that of Coomassie brilliant blue R-250 staining. A trypsin-like **protease** in Pronase, clostripain as a contaminant in a commercial preparation of **Clostridium** histolyticum collagenase, and cysteine **proteases** in Porphyromonas gingivalis could be detected.

L16 ANSWER 10 OF 11 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 2002313187 EMBASE
TITLE: Rapid determination of substrate specificity of **Clostridium** histolyticum .beta.-collagenase using an immobilized peptide library.
AUTHOR: Hu Y.; Webb E.; Singh J.; Morgan B.A.; Gainor J.A.; Gordon T.D.; Siahaan T.J.
CORPORATE SOURCE: T.J. Siahaan, Dept. of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045, United States. siahaan@ku.edu
SOURCE: Journal of Biological Chemistry, (8 Mar 2002) 277/10 (8366-8371).
Refs: 21
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The molecular basis of the substrate specificity of **Clostridium** histolyticum .beta.-collagenase was investigated using a combinatorial **method**. An immobilized positional peptide library, which contains 24,000 sequences, was constructed with a 7-hydroxycoumarin-4-propanoyl (Cop) fluorescent group attached at the N terminus of each sequence. This immobilized peptide library was incubated with C. histolyticum .beta.-collagenase, releasing fluorogenic fragments in the solution phase. The relative substrate specificity (k(cat)/K(m)) for each member of the library was determined by measuring **fluorescence** intensity in the solution phase. Edman sequencing was used to assign structure to subsites of active substrate mixtures. Collectively, the substrate preference for subsites (P(3)-P(4)') of C. histolyticum .beta.-collagenase was determined. The last position on the C-terminal side in which the identity of the amino acids affects the **activity** of the enzyme is P(4)', and an aromatic side chain is preferred in this position. The optimal P(1)'-P(3)' extended substrate sequence is P(1)'-Gly/Ala, P(2)'-Pro/Xaa, and P(3)'-Lys/Arg/Pro/Thr/Ser. The Cop group in either the P(2) or P(3) position is required for a high substrate **activity** with C. histolyticum .beta.-collagenase. S(2) and S(3) sites of the **protease** play a dominant role in fixing the substrate specificity. The immobilized peptide library proved to be a powerful approach for assessing the substrate specificity of C. histolyticum .beta.-collagenase, so it may be applied to the study of other **proteases** of interest.

L16 ANSWER 11 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 2002:239678 SCISEARCH

THE GENUINE ARTICLE: 528WR
TITLE: Rapid determination of substrate specificity of **Clostridium** histolyticum beta-collagenase using an immobilized peptide library
AUTHOR: Hu Y B; Webb E; Singh J; Morgan B A; Gainor J A; Gordon T D; Siahaan T J (Reprint)
CORPORATE SOURCE: Univ Kansas, Dept Pharmaceut Chem, Lawrence, KS 66045 USA (Reprint); Sterling Winthrop Res Inst, Collegeville, PA 19426 USA
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (8 MAR 2002) Vol. 277, No. 10, pp. 8366-8371.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 21

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The molecular basis of the substrate specificity of **Clostridium** histolyticum beta-collagenase was investigated using a combinatorial **method**. An immobilized positional peptide library, which contains 24,000 sequences, was constructed with a 7-hydroxycoumarin-4propanoyl (Cop) fluorescent group attached at the N terminus of each sequence. This immobilized peptide library was incubated with C. histolyticum beta-collagenase, releasing fluorogenic fragments in the solution phase. The relative substrate specificity (k(cat)/K-m) for each member of the library was determined by measuring **fluorescence** intensity in the solution phase. Edman sequencing was used to assign structure to subsites of active substrate mixtures. Collectively, the substrate preference for subsites (P-3-P-4') of C. histolyticum,B-collagenase was determined. The last position on the C-terminal side in which the identity of the amino acids affects the **activity** of the enzyme is P-4', and an aromatic side chain is preferred in this position. The optimal P-1'-P-3' extended substrate sequence is P-1'-Gly/Ala, P-2'-Pro/Xaa, and P-3'-Lys/Arg/Pro/Thr/Ser. The Cop group in either the P-2 or P-3 position is required for a high substrate **activity** with C. histolyticum beta-collagenase. S-2 and S. sites of the **protease** play a dominant role in fixing the substrate specificity. The immobilized peptide library proved to be a powerful approach for assessing the substrate specificity of C histolyticum beta-collagenase, so it may be applied to the study of other **proteases** of interest.

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